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DOCUMENT-IDENTIFIER: US 6296843 B1
TITLE: Mutagenized IL 13-based chimeric molecules

ABPL:
This invention provides mutagenized interleukin 13 molecules that show improved specificity for the restricted (IL4 independent) IL13 receptor and reduced cross-reactivity with the IL4/IL4 shared receptor. The mutagenized IL13 molecules include one or more mutations in a domain that interacts with the 140 kDa hIL4R.beta. or the hIL13R.alpha..sup.1 subunit. These mutagenized IL13 molecules provide effective targeting moieties in chimeric molecules (e.g. fusion proteins) that specifically deliver effector molecules (e.g. cytotoxins) to cells overexpressing IL13 receptors (e.g. cancer cells such as gliomas).

BSPR:
In particular, where the effector component is a cytotoxin, the chimeric molecule may act as a potent cell-killing agent specifically targeting the cytotoxin to cells bearing a particular target molecule. For example, chimeric fusion proteins which include interleukin 4 (IL4) or transforming growth factor (TGF.alpha.) fused to Pseudomonas exotoxin (PE), interleukin 2 (IL2) fused to Diphtheria toxin (DT) have been shown to specifically target and kill cancer cells (Pastan et al., Ann. Rev. Biochem., 61: 331-354 (1992)).

BSPR:
This invention provides novel targeting ligands (specific binding moieties) that have increased specificity for cancer cells as compared to normal cells and therefore extremely effective for specifically delivering effector molecules to various neoplasias. The targeting ligands are mutagenized IL13 molecules having one or more mutations in the domain that interacts with the hIL4 receptor subunit designated the 140 kDa hIL4R.sub..beta. subunit.

BSPR:
Particularly preferred mutagenized IL13 molecules (specific binding moieties) of this invention are mutagenized human IL13 molecules. These molecules can be mutated at one or more of a variety of residues including at residues 12, 13, 14, 65, 66, 67, 68, 69, 70, 109, or 112. In one particular embodiment, residue 13 is a basic amino acid. Other preferred mutagenized IL13 molecules include lysine or arginine at residue 13 and/or aspartic acid at residue 66 and/or aspartic acid at residue 69 and or aspartic acid at residue 109 or 112. When a native human IL13 is mutated these mutations can include hIL13.E13R, hIL13.R66D, hIL13.S69D, hIL13.E13K, hIL13.R109D, and hIL13.R112D. Preferred double mutations include hIL13.E13K/R66D or hIL13.E13K/S69D. The specific binding moieties (mutagenized IL13) can be attached to and therefore comprise an effector molecule as described herein.

BSPR:
In one embodiment, any of the mutagenized IL13 molecules described herein is a component of a chimeric molecule having the formula:

BSPR:
Preferred cytotoxic chimeric molecules are fuision proteins and include any of the mutagenized IL13 molecules described herein fused to the cytotoxin. Particularly preferred cytotoxins include any of the above mutagenized IL13

molecules fused to a Pseudomonas exotoxin (e.g., hIL13.E13K-PE38QQR, hIL13.E13K-PE4E, etc.). Particularly preferred cytotoxic molecules include, but are not limited to hIL13.E13K-PE38QQR, hIL13.E13K-PE4E, hIL13.R66D-PE38QQR, hIL13.R66D-PE4E, hIL13.S69D-PE38QQR, hIL13.S69D-PE4E, hIL13.R109D-PE38QQR, hIL13.R112D-PE38QQR, hIL13.R109D-PE4E, hIL13.R112D-PE4E hIL13.E13K/R109D-PE38QQR, hIL13.E13K/R112D-PE38QQR, hIL13.E13K/R66D-PE4E, hIL13.E13K/RS69D-PE4E, DT390-hIL13.E13K, DT390-hIL13.R66D, DT390-hIL13.S69D, DT390-hIL13.R109D, DT390-hIL13.R112D, DT390-hIL13.E13K/R109D, and DT390-hIL13.E13K/R112D.

BSPR:
In another embodiment, this invention provides methods of delivering an effector molecule to a cell bearing an interleukin 13 receptor (IL13R). The methods involve contacting the cell with a chimeric molecule comprising the effector molecule attached to any of the mutagenized interleukin 13 (IL13) molecules described herein. The methods can involve any of the chimeric molecules described herein.

BSPR:
Where the effector molecule is a cytotoxin, this invention provides methods of killing a cell or inhibiting the growth (and/or proliferation) of a cell expressing an IL13 receptor (IL13R). Again, these methods involve contacting the cell any of the mutagenized IL13-cytotoxin chimeric molecules described herein. In a preferred embodiment, the cell is a neoplastic cell (e.g. a glioma).

BSPR:
The mutagenized IL13 can also be attached to a detectable label. The chimeric label can be used to detect and/or localize and/or quantify a cell or cells expressing an IL13 receptor. The label when administered to a subject will localize at the site(s) of cells expressing or overexpressing IL13 receptors and detection of the label provides an indication of the presence, absence, quantity or location of such cells. Similarly ex vivo detection can be accomplished e.g. using a biological sample taken from the subject.

BSPR:
This invention also provides kits for the detection of cells expressing IL13 receptors or for inhibiting the growth and/or proliferation of such cells. The kits preferably include one or more containers containing a mutagenized IL13 of this invention. The mutagenized IL13 can be attached to either label (e.g. for detection of an IL13R bearing cell) or a cytotoxin (e.g. for inhibiting the growth of an IL13R bearing cell). Any of the cytotoxic or label (or other) chimeric molecules of this invention can be included in the kit.

BSPR:
The term "specifically binds", as used herein, when referring to a protein or polypeptide, or receptor refers to a binding reaction which is determinative of the presence of the protein or polypeptide or receptor in a heterogeneous population of proteins and other biologics. Thus, under designated conditions (e.g. immunoassay conditions in the case of an antibody), the specified ligand or antibody binds to its particular "target" (e.g an IL13 specifically binds to an IL13 receptor) and does not bind in a significant amount to other proteins present in the sample or to other proteins to which the ligand or antibody may come in contact in an organism.

BSPR:
The hIL4 receptor subunit designated the 140 kDA hIL4R.sub..beta. subunit refers to a polypeptide that is common to a shared IL13/IL4 receptor and all other IL4 receptors on "normal" (non-neoplastic cells) such as HUVEC (endothelial cells) (see, e.g., Idzerda et al. (1990) J. Exp. Med., 171: 861-873).

BSPR:
The phrase "a domain that interacts (or specifically interacts) with the hIL13/IL4 receptor subunit designated the 140 kDA hIL4R.sub..beta. subunit", as used herein, refers to a domain of a polypeptide (e.g. IL13) disruption of which reduces or eliminates binding of an IL13 to the IL13/IL4 receptor or that

reduces or eliminates effector activity (e.g. cytotoxic activity) of a chimeric molecule having the disrupted IL13 molecule on a cell or cells that express the 140 kDa hIL4R.beta., subunit (e.g., HUVEC endothelial cells). Alteration of one or more amino acids in the domain preferably diminishes or eliminates interaction with cells expressing the 140 kDA hIL4R.sub..beta. subunit but shows improvement in the interaction of the IL13 or IL13 chimeric molecule on cells over-expressing restrictive IL4R-independent IL13 binding sites (e.g., on gliomas).

BSPR:
A mutation in a polypeptide refers to the substitution of an amino acid at a particular position in a polypeptide with a different amino acid at that position. Thus, for example, the mutation hIL13.E13K indicates that the native amino acid at position 13 in IL13 (glutamic acid, E) is replaced with lysine (K). The "mutation" does not require an actual removal and substitution of the amino acid(s) in question. The protein can be created de novo with the "replacement" amino acid in the position(s) of the desired mutation(s) so the net result is equivalent to the replacement of the amino acid in question.

BSPR:
A "mutagenized IL13 " or "mutagenized hIL13" refers to an IL13 in which one or more of the amino acids differ from the corresponding amino acids in the native form of the IL13. Thus, for example, where a native human IL13 has a glutamic acid at position 13, a mutagenized human IL13 can have an amino acid other than glutamic acid at position 13 (e.g., glutamic acid is substituted with lysine). It will appreciated that mutagenized IL13 molecules of this invention include mutagenized IL13 molecules of other mammalian species (e.g., rat, murine, porcine, largomorph, non-human primates, bovine, canus, and the like) and this invention contemplates the use of chimeric molecules in veterinary as well as human medical conditions.

BSPR:
A chimeric molecule, as used herein refers to a molecule in which, two or more molecules that exist separately in their native state are joined together to form a single entity (molecule) having the desired functionality of all of its constituent molecules. Preferred chimeric molecules of this invention involve one or more IL13 (more preferably a mutagenized human IL13) joined to one or more effector molecules. The mutagenized IL13 acts as a targeting molecule preferably binding the chimeric molecule to cells expressing or overexpressing a restrictive IL4R-independent IL13 receptor (IL13R).

BSPR:
A "specific binding moiety" or a "targeting moiety" refers to a molecule (e.g., a polypeptide) that specifically binds to a particular target. Thus, for example, an interleukin-13 (IL13) is a specific binding moiety that specifically binds to an IL13 receptor (although it will be recognized that where the IL13 receptor shares a component with an IL4 receptor) the specific binding moiety may cross-react with the IL4 receptor. Nevertheless the binding moiety is still regarded as specific because its interaction is specific to these two components and it does not generally bind to any protein found in the organism or biological sample. Preferred specific binding moieties of this invention preferentially bind to the restrictive IL4R-independent tumor associated IL13 receptor rather than the IL4 receptor and typically show an avidity and/or specificity for an IL13 receptor at least 1.5-fold, preferably at least 2-fold, more preferably at least 5-fold, and most preferably at least 10-fold or even at least 100-fold greater than its affinity and/or specificity for an IL4 receptor.

BSPR:
The term "delivering an effector molecule to a cell" refers to preferentially binding such that when an organism is systemically treated with a chimeric molecule comprising a mutagenized IL13 of this invention, or when a cell culture is treated with a chimeric molecule comprising a mutagenized IL13 of this invention, the chimeric molecule preferentially accumulates adjacent to or on the target cell or is preferentially internalized by the cell as compared to

cells lacking or having a lower concentration of the target to which the mutagenized IL13 is directed (e.g. the IL13 receptor).

BSPV:
in which R.sup.1 is the mutagenized human interleukin 13, j and n are independently 0 or 1; R.sup.2 is an effector molecule; and L is an optional linker. The effector molecule can be virtually any molecule that can be attached to the mutagenized IL 13. Effector molecules include, but are not limited to cytotoxins, labels, antibodies, liposomes, lipids, DNA or RNA nucleic acids, DNA or RNA vector, recombinant viruses, chemotherapeutics, anti-cancer antibiotics, photosensitizers, and the like. Particularly preferred cytotoxins include a Pseudomonas exotoxin or a Diphtheria toxin. The Pseudomonas exotoxin can be modified such that it substantially lacks domain Ia, and most preferred Pseudomonas exotoxins include PE38QQR and PE4E. It will be appreciated that the effector molecule can be attached to either the amino terminus, the carboxyl terminus or to an internal residue of the mutagenized IL13 molecule although terminal attachment is preferred.

DEPR:
This invention provides ligands that are highly specific to the IL13 receptor and, when incorporated into chimeric molecules (e.g., chimeric fusion proteins) are capable of specifically directing the chimeric molecules to cells expressing IL13 receptors. Since IL13 receptor targets are characteristically overexpressed on cancer cells, the targeting agents of this invention are particularly useful for specifically directing agents to those cancer cells (e.g., gliomas). In a preferred embodiment, the ligands are mutagenized IL13 molecules, in particular, IL13 molecules containing one or more mutations in a domain that interacts with the hIL13/hIL4 receptor subunit designated the 140 kDA hIL4R.sub..beta. subunit.

DEPR:
The target IL13 receptors are growth factor receptors that show a number of similarities to the IL4 receptors. Studies of the similarities and differences between the IL13 receptor (IL13R) and the IL4 receptor (IL4R) suggest that IL13 binds to the IL4 receptor (as well as to the IL13 receptor) and that IL13 binding to the IL4 receptor is fully competed for by IL4 (Zurawski et al. (1995) J. Biol. Chem., 270: 13869-13878, Vita et al. (1995) J. Biol. Chem., 270: 3512-3517, and Tony et al. (1994) Eur. J. Biochem., 225: 659-665)).

DEPR:
A recently proposed model for the hunan IL13 receptor suggests that it is heterodimeric and comprises an hIL13 binding protein (Debinski et al. (1995) J. Biol. Chem., 270: 16775-16780), Obiri et al. (1995) J. Biol. Chem. 270: 8797-8804, Caput et al. (1996) J Biol. Chem. 271: 16921-16926; Hilton et al. (1996) Proc. Natl. Acad. Sci., USA, 93: 497-501) and a 140 kDa hIL4R.sub..beta. (Obiri et al. (1996) Clin. Cancer Res. 2: 1743-1749) (FIG. 1). The latter is a subunit shared with the hIL4 receptor (Debinski et al. (1996) J. Biol. Chem., 271: 22428-22433, Zurawski et al. (1995) J. Biol. Chem., 270: 13869-13878, Vita et al. (1995) J. Biol. Chem., 270: 3512-3517, and Tony et al. (1994) Eur. J. Biochem., 225: 659-665, Hilton et al. (1996) Proc. Natl. Acad. Sci. USA, 93: 497-50 1). Thus, human interleukin-13 (hIL13) may contain at least two receptor interaction sites (domains): (i) one which recognizes the 140 kDa hIL4R.sub..beta. subunit, and (ii) another site which interacts with its proper binding proteins (Obiri et al. (1995) J. Biol. Chem. 270: 8797-8804, Caput et al. (1996) J Biol. Chem. 271: 16921-16926; Hilton et al. (1996) Proc. Natl. Acad. Sci., USA, 93: 497-501). These putative sites are proposed here based on structural homology to hIL4 and the belief that hIL13 exists as a compact core-bundle of the four anti-parallel a-helices cytokine (FIG. 2).

DEPR:
A predictive model of hIL13 is described by Bamborough et al. (1994) Prot. Eng., 7: 1077-1082. This model is analogous to the model of hIL4 which proposes that IL4 binds the 140 kDa hIL4R.sub..beta. through one site and the .gamma..sub.c , subunit through another site which produces heterodimeric high affinity hIL4R (Russell et al. (1993) Science, 262: 1880-1883). A mutation of glutamic acid at

position 9 to lysine in hIL4 (hIL4.E9K) severely impairs binding of hIL4 to the 140 kDa hIL4R.sub..beta. (Kruse et al. (1993) EMBO J. 12: 5121-5129) (FIG. 3).

DEPR:
Recently, it was demonstrated that human (h) gliomas express large number of receptors (R) for interleukin 13 (IL13) (Debinski et al. (1995) Clin. Cancer Res. 1: 12531-1258). It was also shown that both IL4 and an antagonist of hIL4, hIL4.Y124D, which binds the 140 kDa hIL4R .beta.-chain protein and block the effects of hIL13 and hIL4 on normal cells, did not block the binding and internalization of IL13 in glioma cells unlike on normal cells and some adenocarcinomas (Debinski et al. (1995) Clin. Cancer Res. 1: 1253-1258; Debinski et al. (1996) J. Biol. Chem. 271: 22428-224; Debinski et al. (1995) J. Biol. Chem. 270; 16775-16780). These and other findings demonstratc the existence of hIL13 receptors (e.g., on cancers) that do not interact with IL4 and presumably do not involve the 140 kDa hIL4R .beta.-chain (hIL4R.beta.)

DEPR:
This was demonstrated by the observation that the use of hIL4 and hIL4.Y124D in conjunction with IL13R directed chimeric molecules enhanced the specificity of these molecules to cells bearing the IL13 receptor.

DEPR:
It is demonstrated herein that a similar effect to that exhibited by hIL4 and hIL4.Y124D can be obtained by mutagenizing IL13 itself and using the mutagenized IL13 as a targeting moiety in a chimeric molecule. In one embodiment, cytotoxins are described herein in which the targeting moiety (IL13) is mutagenized by changing glutamic acid at position 13 to lysine (producing hIL13.E13K) and the toxic effector molecule is a Pseudomonas exotoxin A (PE) derivative (e.g., PE38QQR, or PE4E).

DEPR:
It is also taught herein that by altering a putative binding site of IL13 which interacts with the 140 kDa IL4 receptor .beta.-chain, one can alter interaction of the cytotoxins (or other IL13R-directed chimeric molecules) with the IL13R and IL4R common elements that are predominantly expressed in normal tissues. Indeed, it is demonstrated herein that, for example, hIL13.E13K-PE4E is less active on normal cells, such as endothelial cells, which do express elements common to both hIL4 and hIL13R. Unexpectedly, the action of hIL13.E13K-PE4E was considerably more potent on human glioma cells when compared with that containing the wild-type hIL13. Toxicities of the hIL13.E13K-based cytotoxins in vivo are also several times lower when compared with chimeric cytotoxins utilizing unmutagenized hIL13 as a targeting moiety. Thus, it is demonstrated herein that a mutation in the domain of IL13 that interacts with the hIL4 receptor subunit designated the 140 kDa hIL4R.sub..beta. subunit (e.g., a mutation at IL13 residue 13) makes a chimeric cytotoxin less active on normal cells and, surprisingly, much more active on glioma cells. The increase in an overall specific cytotoxic activity can be as high as 100-fold. Thus, hIL13 is amenable to engineering which leads to a much more discriminate recognition of the hIL13R that is expressed on cancer cells from the one present on normal cells.

DEPR:
As explained below, in a preferred embodiment the mutagenized IL13 can be provided as a component of a chimeric molecule. Alternatively, the mutagenized IL13 may be provided alone to bind to and thereby specifically block the IL13 receptor.

DEPR:
Using the mutagenized IL13 molecules, this invention provides in one embodiment, methods for specifically delivering an effector molecule to a cell bearing an IL13 receptor (e.g., a tumor cell such as a glioma). These methods utilize chimeric molecules comprising a mutagenized IL13 (targeting molecule) attached to an effector molecule. The chimeric molecules of this invention specifically target tumor cells (especially glioma cells) while providing reduced binding to non-target cells as compared to other targeted chimeric molecules known in the